Patent Questionnaire PF261

1. With respect to the DNA sequence:

   a. Is it full length? (please type YES or NO): YES

   b. In Figure 1 of the patent application, show the full protein sequence with nucleotide correspondence. *Underline* putative leader sequences.

2. Has the protein been expressed? (please type YES or NO): YES

   If Yes, answer the following questions:

   If the protein has not been expressed, provide the following information as if you were to express the protein.

   a. Was a bacterial expression system used? (please type YES or NO):

   What is the size of the protein? ~~[illegible]~~ GST 31 KDa

   What vector was used? PD10

   What host was used? M15 rep 5

   What were the primer sequences?

   5' primer?
   GCG GCG GGA TCC ATG GCT ATG ATG GAG GTC CAG

   3' primer?
   CGC GCG TCT AGA GCT TAG GCA ACT AAA AAG GCC

   Did the gene encode a "tag" for purification? Explain:

   Yes ~~NO~~ - PD10 has a 5' Hexa His Tag in Vector

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   If the protein has not been expressed, provide the following information as if you were to express the protein.

   a. Was a bacterial expression system used? (please type YES or NO):

   What is the size of the protein? ~~[illegible]~~ GST 31 KDa

   What vector was used? PDIO

   What host was used? M15 rep 5

   What were the primer sequences?

   5' primer?
   GCG GCG GGA TCC ATG GCT ATG ATG GAG GTC CAG

   3' primer?
   CGC GCG TCT AGA GCT TAG GCA ACT AAA AAG GCC

   Did the gene encode a "tag" for purification? Explain:

   Yes ~~[illegible]~~ - PDIO has a 5' Hexa-His Tag in Vector

Ruben EXHIBIT 2105
Ruben v. Wiley et al.
Interference No. 105,077
RX 2105

Provide a Figure of the expressed protein in the application.

3' primer?

b. Will a different expression system be used? Explain.
The gene was put into the baculovirus expression system. Two different constructs were made at different methionines on the 5' end of the open reading frame. (See ABOVE)

3. Was the protein renatured or modified to produce active protein?
(please type YES or (NO):

If Yes, please explain:

4. Therapeutic/Diagnostic Applications for Protein: See attachment

a. Can the protein be used to identify a receptor? (please type (YES) or NO):
Please attach an appropriate literature reference where a similar protein was used to identify a receptor.

b. Can the protein be used to identify a ligand? (please type YES or (NO)):
If Yes, please attach an appropriate literature reference where a similar protein was used to identify a ligand.

c. Can the protein be used in a screening assay to identify small molecule antagonists or agonists? (please type YES or NO):

Please attach any appropriate literature if available where a similar protein/receptor combination was used in a screening assay. Alternatively, if you could provide a brief description of how one might set up a screening assay, please do so (on a separate page).

d. Would an antibody raised against this protein represent a potential therapeutic agent? (please type YES or NO):

If Yes, please explain: A protein ~~antibody~~ against the ~~protein~~ could prevent the ligand from interacting with the receptor. It may have a therapeutic potential with regard to auto immune diseases.

e. Are there any potential diagnostic uses of this protein? (please type YES or NO):

If Yes, please explain: Variations in the sequence or expression of FASL may serve as potential diagnostic markers for disease states relating to the function of the protein, possibly involving peripheral tolerance or autoimmune disease

6. Was the EST for this invention first identified at TIGR? (please type YES or NO):

7. Please provide the full name (including middle initial), home address and country of citizenship of all all HGS inventors *on a separate page.*

8. If this gene shares homology to previously-published genes, include please include a comparison figure (Amino Acids) in the patent application. This will likely apply to most genes being patented.

9. Did any scientist at SmithKline (or any other organization) contribute in any way to this invention? (If yes, please list contributors)

10. What cDNA library was this sequence isolated from?

Human Pancreas Tumor.

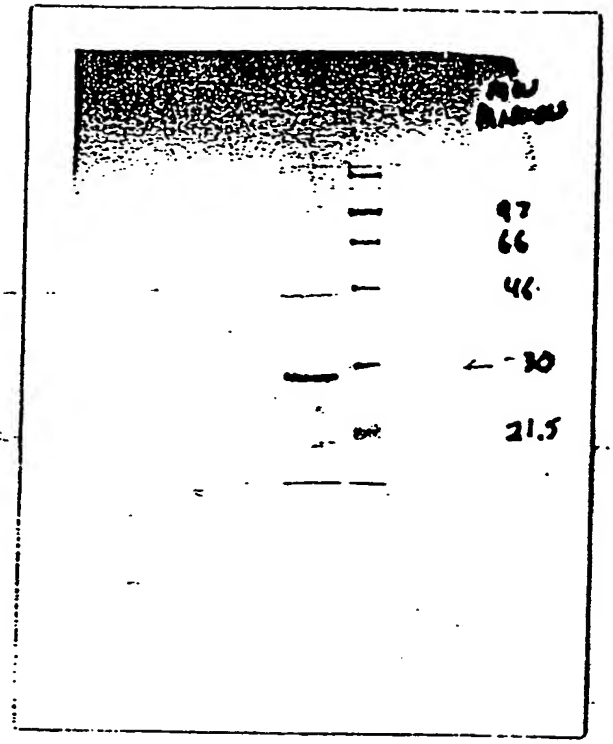
HGS CLONE ID:

25750 HTPAN08 (R) 5-1,743

HGS FULL LENGTH NUMBER:

413412 — 5-1,743 (R)

Upon completion of this questionnaire, turn it in to Regina and let her know when you will be ready to make a deposit to ATCC. This will need to be accomplished as soon as possible once the questionnaire is turned in.


MW
MARKERS
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66
46
30
21.5

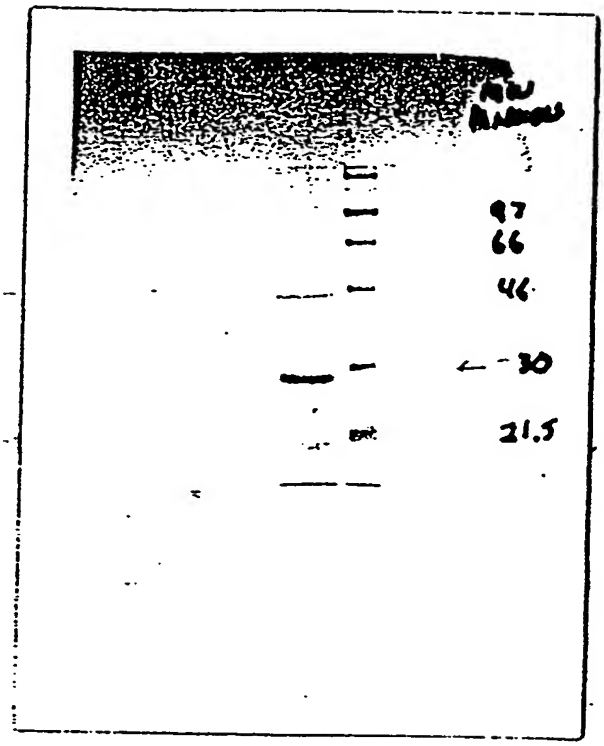
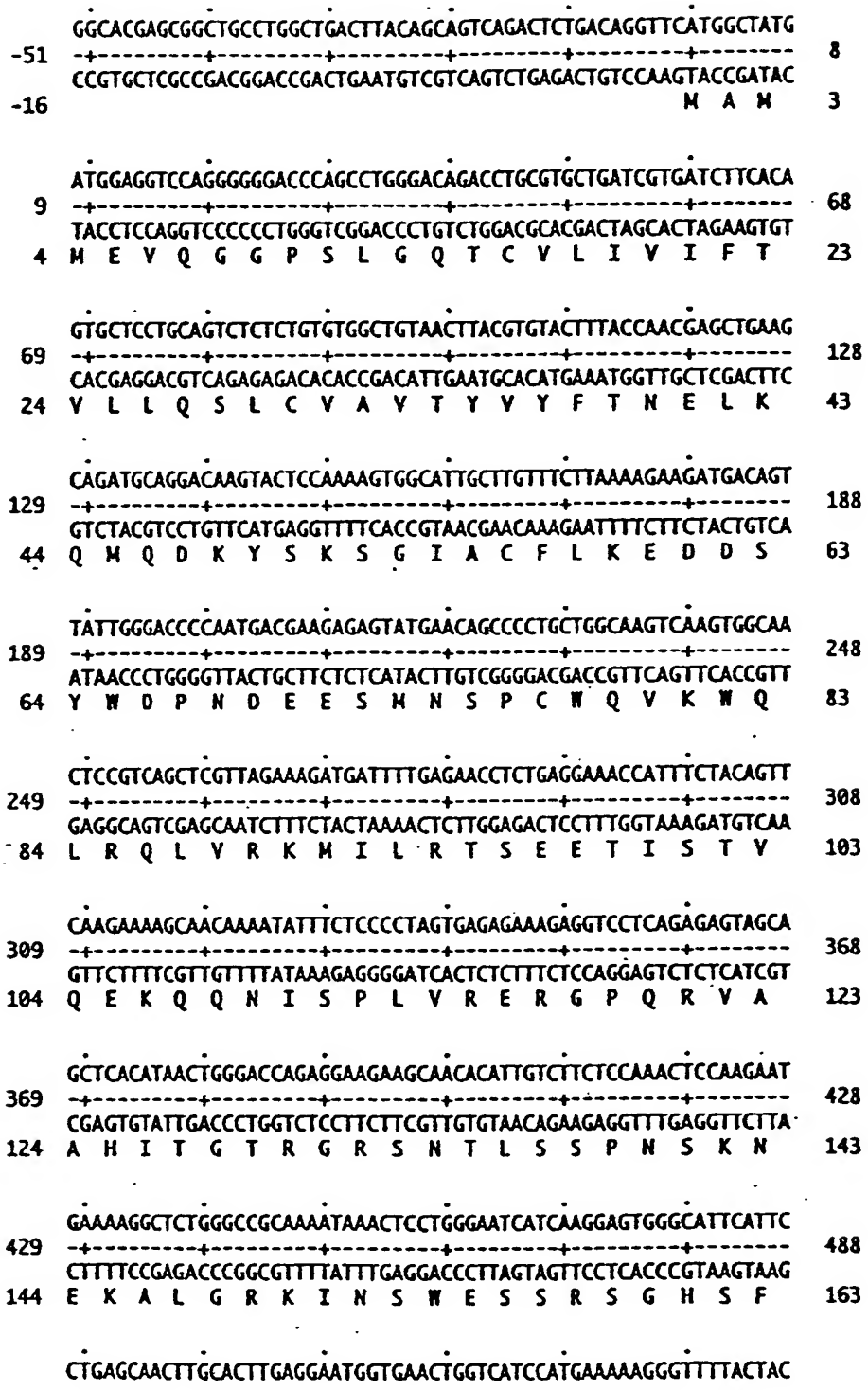
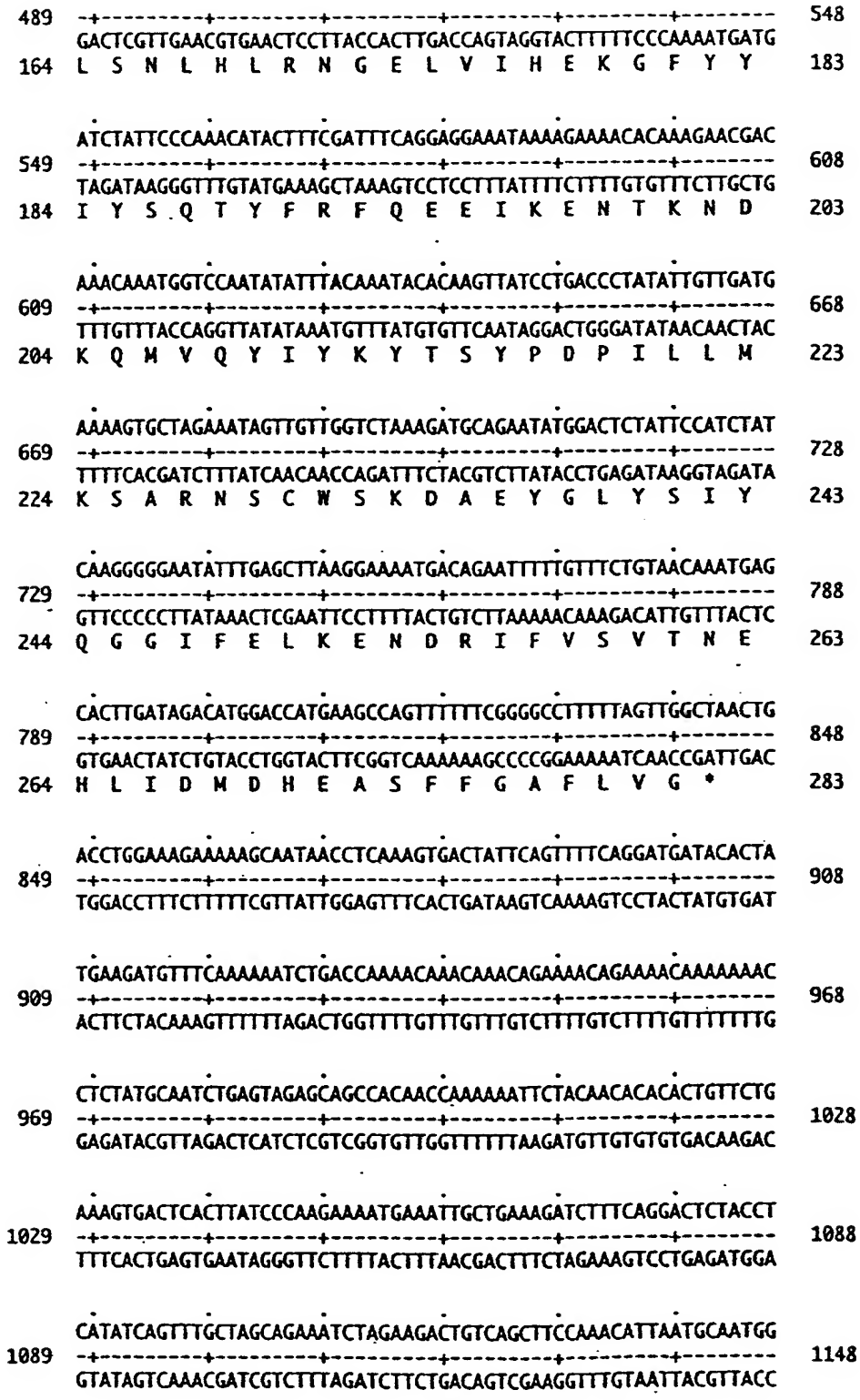

97
66
46
30
21.5

Figure 1. Nucleotide and Amino Acid sequence of Fas Ligand




489 -+---------+---------+---------+---------+---------+-------- 548
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164 L S N L H L R N G E L V I H E K G F Y Y 183
ATCTATTCCCAAACATACTTTCGATTTCAGGAGGAAATAAAAGAAAACACAAAGAACGAC
549 -+---------+---------+---------+---------+---------+-------- 608
TAGATAAGGGTTTGTATGAAAGCTAAAGTCCTCCTTTATTTTCTTTTGTGTTTCTTGCTG
184 I Y S Q T Y F R F Q E E I K E N T K N D 203
AAACAAATGGTCCAATATATTTACAAATACACAAGTTATCCTGACCCTATATTGTTGATG
609 -+---------+---------+---------+---------+---------+-------- 668
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224 K S A R N S C W S K D A E Y G L Y S I Y 243
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729 -+---------+---------+---------+---------+---------+-------- 788
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244 Q G G I F E L K E N D R I F V S V T N E 263
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789 -+---------+---------+---------+---------+---------+-------- 848
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264 H L I D M D H E A S F F G A F L V G * 283
ACCTGGAAAGAAAAAGCAATAACCTCAAAGTGACTATTCAGTTTTCAGGATGATACACTA
849 -+---------+---------+---------+---------+---------+-------- 908
TGGACCTTTCTTTTTCGTTATTGGAGTTTCACTGATAAGTCAAAAGTCCTACTATGTGAT
TGAAGATGTTTCAAAAAATCTGACCAAAACAAACAAACAGAAAACAGAAAACAAAAAAAC
909 -+---------+---------+---------+---------+---------+-------- 968
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969 -+---------+---------+---------+---------+---------+-------- 1028
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AAAGTGACTCACTTATCCCAAGAAAATGAAATTGCTGAAAGATCTTTCAGGACTCTACCT
1029 -+---------+---------+---------+---------+---------+-------- 1088
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1089 -+---------+---------+---------+---------+---------+-------- 1148
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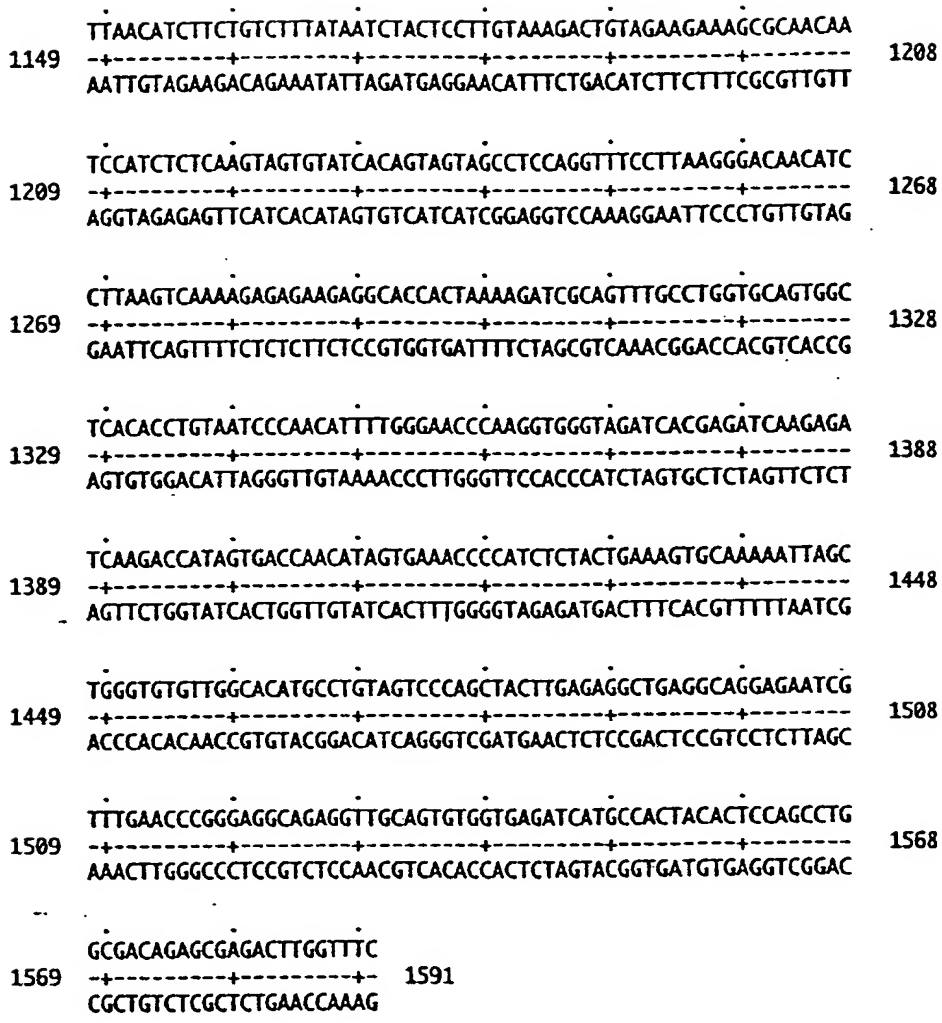
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1388
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TCAAGACCATAGTGACCAACATAGTGAAACCCCATCTCTACTGAAAGTGCAAAAATTAGC
1389
1448
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1508
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1509
1568
AAACTTGGGCCCTCCGTCTCCAACGTCACACCACTCTAGTACGGTGATGTGAGGTCGGAC
GCGACAGAGCGAGACTTGGTTTC
1569
1591
CGCTGTCTCGCTCTGAACCAAAG

Figure 2. Alignment of Fas ligand to Human Fas Ligand

Percent Similarity: 48.594 Percent Identity: 22.892

faslpep.pep x faslhuman.pep

```
  4 MEVQGGPSLGQTCVLIVIFTVL.................LQSLCVAVTYV 36
    ::  ::..::....::.. | :                 |.. ..:....
 15 vdssasspwappgtvlpcptsvprrpgqrrpppppppppplpppppppplp 64

 37 YFTNELKQMQDKYSKSGIACFLKEDDSYWDPNDEESMNSPCWQVKWQLRQ 86
     :.  |..:..: ..|.  |:|     :    .  ::. . :|: ::|..
 65 plp..lpplkkrgnhstglcllvm..ffmvlvalvglglgmfql.fhlqk 109

 87 LVRKMILRTSEETISTVQEKQQNISPLVRERGPQRVAAHITGTRGRSNTL 136
     : .:  .||:  ...  ||| . .. ..|: . | .||:|   |:||.
110 elaelrestsqmhtasslekqighpspppekkelrkvahlt...gksnsr 156

137 SSPNSKNEKALGRKINSWESSRSGHSFLSNLHLRNGELVIHEKGFYYIYS 186
    | | ..::.              |  :||.:..:.|:|||:|.|:|::||
157 smplewedty.............givllsgvkykkgglvinetglyfvys 193

187 QTYFRFQEEIKENTKNDKQMVQYIYKYTS.YPDPILLMKSARNSCWSKDA 235
    ..|||      :.. |: .: : :|. .| ||:.:::|.: . |: ..:.
194 kvyfr......gqscnnlplshkvymrnskypqdlvmmegkmmsycttgq 237

236 EYGLYSIYQGGIFELKENDRIFVSVTNEHLIDMDHEASFFGAFLV 280
    ::  | | |::|:|...|:::|.|.:  |::::....||| : :
238 mwar.ssylgavfnltsadhlyvnvselslvnfeesqtffglykl 281
```

Mammalian development is dependent on both the proliferation and differentiation of cells as well as programmed cell death which occurs through apoptosis (Walker, et al, *Methods Achiev.Exp.Pathol.*, 13:18, 1988. Apoptosis plays a critical role in the destruction of immune thymocytes that recognize self antigens. Failure of this normal elimination process may play a role in autoimmune diseases (Gammon, et al., *Immunology Today* 12:193, 1991).

Itoh, et al., (*Cell* 66:233, 1991) described a cell surface antigen, FAS/CD23 that mediates apoptosis and is involved in clonal deletion of T-cells . Fas is expressed in activated T-cells, B-cells, neutorophils and in thymus, liver, heart and lung and ovary in adult mice (Watanabe-Fukunaga et al., *J. Immunolo.* 148:1274, 1992) in addition to activated T-cells, B-cells, neutorophils. In experiments where a monoclonal Ab is cross-linked to FAS, apoptosis is induced (Yonehara, et al., *J. Exp. Med*, 169:1747, 1989; Trauth, et al., *Science* 245:301, 1989). In addition, there is an example where binding of a monoclonal Ab to FAS is stimulatory to T-cells under certain conditions (Alderson, et al., *J. Exp. Med* 178:2231, 1993).

Fas antigen is a cell surface protein of relative Mr of 45 Kd. Both human and murine genes for Fas have been cloned by Watanabe-Fukunaga et al., (*J. Immunolo.* 148:1274, 1992) and Itoh, et al., (*Cell*, 66:233, 1991). The proteins encoded by these genes are both transmembrane proteins with structural homology to the Nerve Growth factor/tumor necrose factor receptor superfamily, which includes two TNF receptors, the low affinity nerve growth factor receptorand CD40, CD27, CD30, and OX40.

An abnormal recessive mutation known as lymphoproliferative mutation (lpr) has been observed in mice in which the Fas antigen cannot transduce an apoptosis signal (Watanabe-Fukunage et al., *Nature*, 356:314, 1992). These mice demonstrate accumulation of CD4-CD8-thymocytes in lymph nodes and spleen. Mice carrying this mutation have both lymphadenopathy and autoimmune disease, suggesting the role

Fas in T-cell development. Therefore, Fas-mediated apoptosis may play an imortant role in peripheral tolerance. Fas also apears to be involved in cytotoxic T-cell mediated apoptosis. The presence of Fas on target cells and the presence of Fas ligand on cytotoxic T-cells results in apoptosis of the target cells.

Recently the Fas ligand has been described (Suda, et al., *Cell* 75:1169, 1993). The amino acid sequence indicates that Fas ligand is a type II transmembrane protein belonging to the TNF family. Fas ligand is expressed in splenocytes and thymocytes, consistent with T-cell mediated cytotoxicity. The purified Fas ligand has a Mr of 40 kd.

Another syndrome similar to that found in lpr mice is known as generalized lymphoproliferative disease (gld) signal (Watanahe-Fukunage et al., *Nature*, 356:314, 1992) which maps to a separate chromosomal loci. The mouse Fas ligand has been localized to the gld region of chromosome 1 (Takahashi, et al., *Cell* 76:969, 1994) while Fas antigen has been localized to the lpr locus on chromosome 19. Splenocytes of wild type and gld mice express Fas ligand following activiation. However, gld carries a point mutation and cannot induce apoptosis.

Recently it has been demonstrated that Fas/Fas ligand interactions are required for apoptosis following the activation of T-cells (Ju et al., *Nature*,373:444, 1995; Brunner et al., *Nature*, 373:441, 1995). Activation of T-cells induces both proteins on the cell surface. Subsequent interaction between the ligand and receptor results in apoptosis of the cells. this supports the possible regulatory role for apoptosis induced by Fas/Fas ligand interaction during normal immune responses.

Claims:

- Tool for studying autoimmune disorders and the roles that Fas L may play in self tolerance
- Fas L may be used for identification of a novel receptor

- Studies employing Fas L and anti Fas L antibodies may provide insight into development of self tolerance by the immune system
- Useful a research tool in elucidating the biology of autoimmune disorders including systemic lupus erythematosis, immunoproliferative disease lymphadenopathy (IPL), angioimmunproliferative lymphadenopathy (AIL), rheumatoid arthritis, diabetes, M.S.
- The use of Fas L in treating graft versus host disease
- Developing treatment for disorders mediated by Fas L. Therapeutically effective amount of Fas L administered to a patient with a disorder caused by defective or insufficient amount of Fas L
- Gene Therapy
- Cancer Diagnostic. this gene is found in many tumor cell lines including pancreatic tumor, testes tumor, endometrial tumer, T-cell lymphoma
-

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